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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Wang et al.

Serial No.: 08/333,680 Group Art Unit: 1804

Filed: November 3, 1994 Examiner: Rories

For: NOVEL ADENOVIRAL VECTORS, PACKAGING CELL LINES, RECOMBINANT ADENOVIRUSES, AND METHODS

Attorney Docket No.: 7639-061

DECLARATION OF QING WANG
UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

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Sir:

I, Qing Wang, do declare that:

1. I am a scientist in the Molecular Biology Division of Cell Genesys, Inc., the assignee of the above-identified application. I have extensive experience in the field of molecular biology, particularly as it relates to adenoviral vectors and packaging cell lines, as evidenced by my curriculum vitae, attached hereto as Exhibit A.

2. The following analyses were carried out by me personally, or under my direction and supervision, to determine if an adenoviral packaging cell line, in particular a 293 embryonal kidney cell line, could be engineered to express the E4 early region gene and the E2A gene region, both under the control of an inducible promoter, in addition to the E1 early region gene, and if a such a cell line would survive and grow.

3. To test whether 293 cells can survive while expressing E1 and E4 early region genes and the cytotoxic E2A gene, I constructed the 293-ORF6/E2A cell line by introducing nucleotide sequences encoding the E4 early region gene in

particular, the essential region open reading frame 6 (ORF6), under the control of the mouse α inhibin promoter and nucleotide sequences encoding the E2A gene region under the control of the mouse α inhibin promoter into 293 cells, and assayed for the presence of the E1 and E4 early region genes and the E2A gene region using the Southern blot assays described below. Southern blot analysis is a routinely used technique to identify DNA sequences from a mixture of DNA in particular genomic DNA, in order to detect the presence of a gene of interest. Detection of E1 and E4 early gene regions and E2A in genomic DNA isolated from 293-ORF6/2A cells indicates that these gene regions are present in the cells.

4. The following plasmids were used to introduce the E4-ORF6 early region gene and the E2A gene region into 293 cells: Plasmid pIK6.1MIPα-ORF6 comprises the minimum essential region of the E4-ORF6 early region gene, the ORF6 coding regions from nucleotide 1846 to 2756, as numbered from the right end of the Ad5 genome, linked to 238 base pairs of the inducible mouse α inhibin promoter. Plasmid pIK6.1MIPα-E2A comprises the E2A gene region, from nucleotide 23435 to 24627, as numbered from the left end of the Ad5 genome, linked to 238 base pairs of the inducible mouse α inhibin promoter.

5. The 293-ORF6/E2A cells were constructed as follows: 293 cells were grown as a monolayer in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l glucose (JRH Biosciences, Denver, PA), 10% donor calf serum (Tissue Culture Biologies, Tulare, CA). Cells were seeded at 5 X 10^5 per 10 cm plate. 10 μg of pIK6.1 MIPα-ORF6 and 1 μg of pGEM-pgkNeo.pghpoly plasmid encoding the neomyocin resistant gene, were co-transfected into the 293 cells by calcium phosphate co-precipitation. The resulting G418 resistent clones were screened for the presence of the E4-ORF6 early gene region indicating a positive clone, designated 293-ORF6 cells. The 293-ORF6 cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM), 4.5 g/l glucose (JRH Biosciences,

Denver PA), 10% donor calf serum (Tissue Culture Biologies, Tulare, CA). Cells were seeded at $5x10^5$ per $10-cm^2$ plate 24 hours prior to the transfection. Ten micrograms of pIK6.1MIPα-E2A plasmid (Figure 1) and 1 mg of pIK6.1 HYg.plasmid encoding the Hygromycin resistance gene under the control of the minimum CMV promoter, were co-transfected into 293-ORF6 cells by calcium phosphate co-precipitation. The hygromycin resistant clones were selected, screened by Southern blot analysis (Figure 2) and maintained in normal culture medium.

6. In order to determine the presence of E4-ORF6 and E2A in the transfected cells, eight cell lines were chosen for screening. Southern blot analysis of 293-ORF6/E2A cell lines was conducted as follows: 10 μg of genomic DNA isolated from each cell line was digested with restriction enzymes Hind III and Dra III, electrophoresed on an 0.8% agarose gel and transferred to a nylon membrane. The nylon membrane, or blot, was first probed for E2A with a Bam H1-EcoRV fragment of the pIK6.1MIPα-E2A plasmid, and sequentially reprobed for E4-ORF6 with a fragment of the ORF6 coding region (nucleotides 1846 to 2756), and E1 with a probe comprising a Hind III fragment of E1 early region gene (m.u. 7.7 to m.u. 17.1). The results indicate that the genomic DNA obtained from the 293-ORF6/E2A cell lines contain the coding region of E2A and E4-ORF6 gene regions in addition to the coding region of E1 (Figure 2).

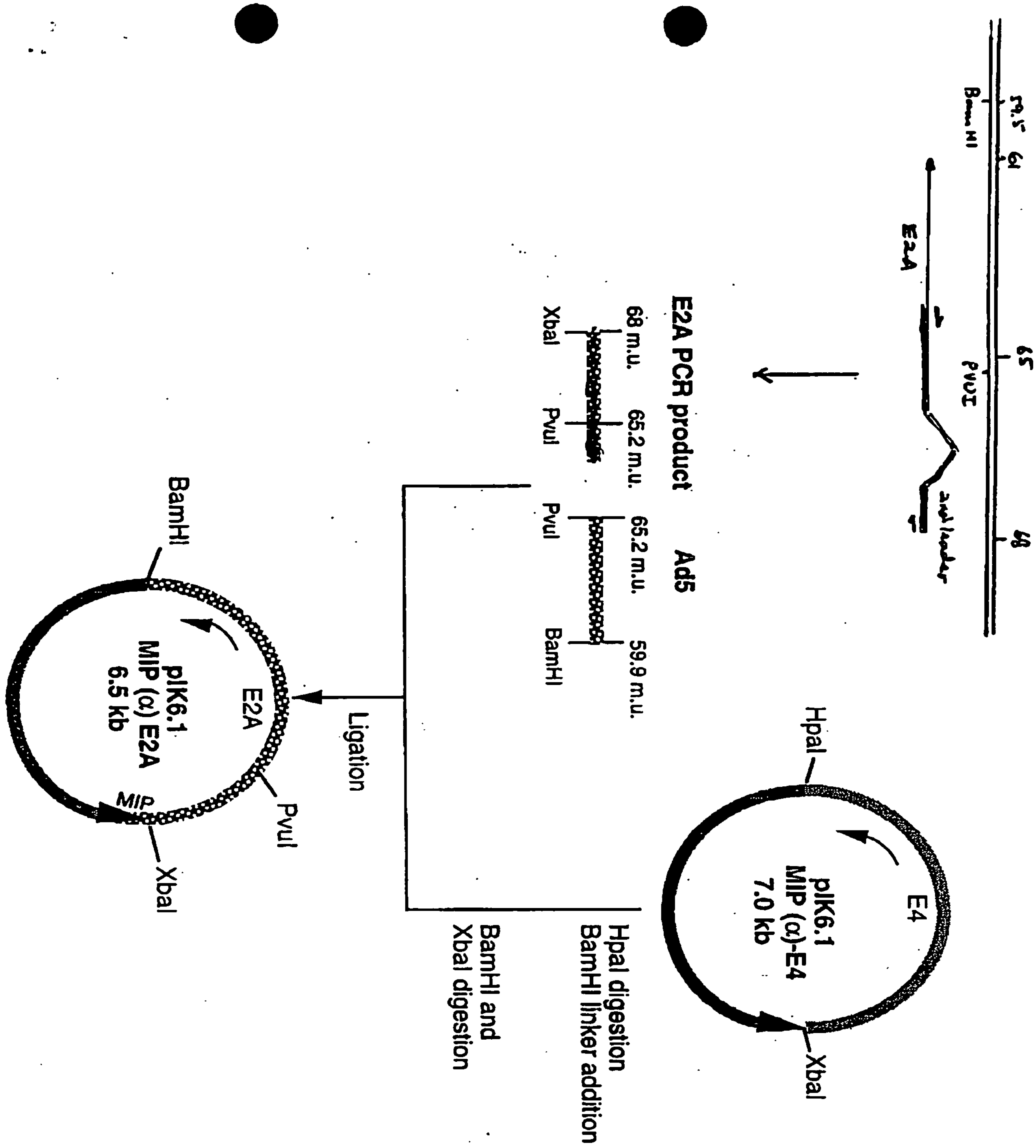
7. The positively identified 293-ORF6/E2A cell lines have been grown in DMEM with 4.5 g/l glucose and 10% donor calf serum, and maintained in culture for at least twelve passages. These cell lines demonstrate similar growth rates and characteristics to those observed by the 293-ORF6 cells. These observations indicate that 293 cells transformed to express both the E4 early region gene and the E2A gene region, in addition to E1 early region gene, are able to survive and grow.

8. The data described in paragraph 6 demonstrates that a copy of the E1 early region gene, the E4-ORF6 gene region and the E2A coding region was successfully detected in the engineered 293-ORF6/E2A cell line indicating presence of the coding regions of E1 and E4 early region genes, in addition to the coding region of E2A. The observations described in paragraph 7 demonstrate that 293 cells transformed to express the E4 early region gene and the E2A gene region, in addition to the E1A early region gene, are able to survive and grow. The results and observations described above demonstrate that the methods taught in the above-identified patent application may be successfully applied to create a cell line expressing E1 and E4 early region genes and E2A under the control of an inducible promoter.

9. I declare further that all statements made in this Declaration of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

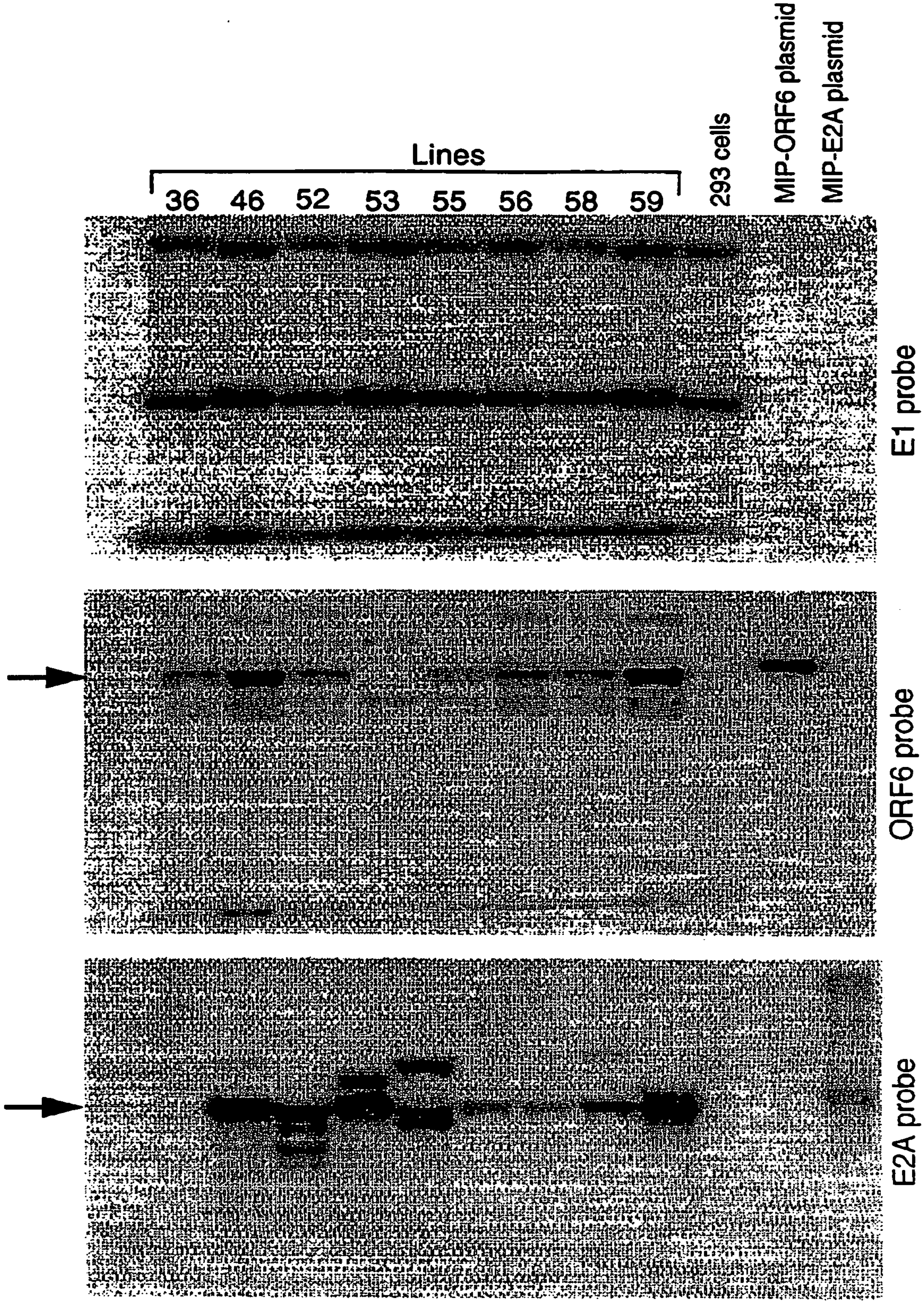
______________________ ______________________________

Date Qing Wang



BamHI
59.5
61
E2A
65
PvuI
2nd leader
68
E2A PCR product
68 m.u.
65.2 m.u.
XbaI
PvuI
Ad5
65.2 m.u.
59.9 m.u.
PvuI
BamHI
HpaI
E4
pIK6.1
MIP (α)-E4
7.0 kb
XbaI
HpaI digestion
BamHI linker addition
BamHI and
XbaI digestion
Ligation
BamHI
E2A
pIK6.1
MIP (α) E2A
6.5 kb
PvuI
MIP
XbaI

FIGURE 1



HindIII + DraIII digestion

FIGURE 2

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EDUCATION

1987-1992	Ph.D. in Molecular Biology & Virology Indiana University, Bloomington, IN
1978-1983	M.D. in medicine Shanghai 2nd Medical University, Shanghai, China (ECFMG certified in US and passed USMLE Licensure Step III Test).

EXPERIENCE

1996-present	Scientist Cell Genesys Inc. Foster City, CA
1995-1996	Associate Scientist Cell Genesys Inc. Foster City, CA.
1992-1994	Postdoctoral Scientist Cell Genesys Inc. Foster City, CA.
1987-1992	Graduate Student and Research Assistant Department of Chemistry and Biology, Indiana University, Bloomington, IN. Thesis: Establishment of adenoviral vector system for efficient gene transfer and gene targeting in mammalian cells.
1985-1986	Resident Pediatrician Shanghai Children's Hospital, Department of Pediatrics, Shanghai 1st Medical University.
1983-1985	Resident Pediatrician Shanghai Xinhua Hospital, Department of pediatrics, Shanghai 2nd Medical University.
1983-1985	Assistant medical researcher Shanghai Pediatric Research Institute.

HONORS AND AWARDS

American Society of Virology 8th Annual Meeting Award (July 9-13, 1989, London, Ontario, Canada).

[redacted]

— Exhibit A Serial No. 08/333,680

American Society of Virology 9th Annual Meeting Award (July 8-12, 1990, Salt Lake City, Utah).

Young Investigator Award. The 7th International & 3rd European Joint Symposium on Purine and Pyrimidine Metabolism in Man. (June 30-July 6, 1991, Bournemouth, England).

PROFESSIONAL AFFILIATION

American Society of Virology

PUBLICATIONS

Wang, Q., Konan V. and Taylor, M.W. (1991), Expression of the *aprt* gene in an adenovirus vector system as a model for studying gene therapy. Purine and pyrimidine Metabolism in Man VII Part B, Plenum Publishing Co. New York, pp. 61-66.

Wang, Q. and Taylor, M.W. (1993), Correction of a mammalian gene by high frequency of homologous recombination using an adenovirus vector. Mol. Cell Biol. 13 (2), 918-927.

Wang, Q., Finer, M.H. and Jia, X-C. (1994), Novel adenoviral vectors, packaging cell lines and methods. (Patent application, WO 96/14061)

Wang, Q., Jia, X-C and Finer, M.H. (1995), A new packaging cell line for propagation of the second generation of the recombinant adenovirus vector. Gene Therapy 2, 775-783.

Wang, Q., & Finer, M.H. (1996), Second-generation adenovirus vectors. Nature Medicine 2:714-716.

Wang, Q., Greenburg, G., Bunch, D., Farson, D., and Finer, M. H. (1997), Persistent transgene expression in vivo mediated by an E1/E4-deleted adenovirus vector. Gene Therapy 4, 393-400.

Wang, Q., Dai, F. and Finer, M.H. Late gene expression and genome stability of a ΔE1/ΔE4 vector: Establishment of 293-ORF6 line to prevent "healing" of E4 deletion (in preparation).

PRESENTATIONS

Persistent transgene expression in vivo using ΔE1/ΔE4 adenovirus vector. Gene Therapy Conference, Cold Spring Harbor, New York, Sept. 24-29, 1996.

Adenovirus vectors for improved in vivo gene delivery. Vaccines: New Technologies & Applications. March 18-20, 1996. Mclean, Virginia

A new packaging cell line for propagation of the second generation of the recombinant adenovirus vector. Fourth International Conference on Gene Therapy of Cancer. Nov. 9-11, 1995. San Diego, CA.

Correction of a CHO *aprt* gene with an adeno/*aprt* vector. American Society for Biochemistry and Molecular Biology/Biophysical Society Joint Meeting. Q. Wang and M.W. Taylor. Feb. 9-13, 1992. Houston, Texas.

Expression of the *aprt* gene in an adenovirus vector system as a model for studying gene therapy. The 7th International/3rd European Joint Symposium on Purine and Pyrimidine Metabolism in Man. Q. Wang, V. Konan and M.W. Taylor. June 30-July 6, 1991. Bournemouth, England.

The regulatory signals on expression of CHO *aprt* gene in target cells. American Society of Virology 9th Annual Meeting. Q. Wang and M. W. Taylor. July 8-12, 1990. Salt Lake City, Utah.

The possible application of adeno-5/*aprt* system on gene therapy. The First Conference. Molecular Biology of APRT. Q. Wang and M. W. Taylor. May 17-20, 1990. Bloomington, Indiana.

An efficient transfer and expression vector system in mammalian cells: Adeno-5/*aprt*. American Society of Virology 8th Annual Meeting. Q. Wang, V. Konan, F. L. Graham and M. W. Taylor. July 9-13, 1989, London, Ontario, Canada.

Efficient expression of a truncated CHO *aprt* gene in adeno-5 vector. The First U.S.-Japan Symposium on Biotechnology. Q. Wang, V. Konan, F. L. Graham and M.W. Taylor. April 7-10, 1989. St. Petersburg Beach, Florida.